Cardiac Stress Protein Elevation 24 Hours After Brief Ischemia or Heat Stress Is Associated With Resistance to Myocardial Infarction

Michael S. Marber, MRCP; David S. Latchman, PhD;
J. Malcolm Walker, MD, FRCP; Derek M. Yellon, PhD, FACC

Background. To test the hypothesis that the heat shock response is associated with myocardial salvage, the heat stress protein (HSP) content of cardiac tissue was increased by either ischemic or thermal stress.

Methods and Results. Rabbits were divided into four groups. Ischemic pretreatment (n=15) comprised four 5-minute episodes of coronary ligation separated by 10 minutes of reperfusion. The corresponding control group (n=21) underwent surgical preparation without coronary ligation. Thermal pretreatment (n=16) involved whole-body temperature elevation to 42°C for 15 minutes; corresponding controls (n=15) were treated with anesthetic alone. Twenty-four hours later, hearts were removed for HSP estimation or infarct size assessment after a 30-minute coronary ligation. Myocardial HSP72 content assessed by Western blotting was elevated by both ischemic and thermal pretreatments (2.5±0.2 units, n=4, and 2.8±0.3 units, n=4, mean±SEM; P=NS, respectively) compared with the corresponding control groups (1.0±0.3, n=4, P<.01 and 0.3±0.1, n=4, P<.01, respectively). HSP60 was preferentially elevated by ischemic pretreatment. After a 30-minute coronary occlusion and 120 minutes of reperfusion, ischemic and thermal pretreatments limited infarct size as a percentage of the volume at risk by 28.8±5.2% vs 52.0±5.2%, P<.01 and 32.8±3.8% vs 56.9±6.5%, P<.01, respectively.

Conclusions. Myocardial stress protein induced by either sublethal thermal or ischemic injury is associated with myocardial salvage. Our findings suggest that stress protein elevation, rather than the nonspecific effects of thermal or ischemic stress, may be responsible for the myocardial protection seen in this model. Our observations may have important implications regarding myocardial adaptation to brief periods of ischemia. (Circulation. 1993;88:1264-1272.)

KEY WORDS • proteins • ischemia • myocardium • reperfusion

When any living cell is exposed to a sublethal elevation of environmental temperature, a series of adaptive changes occur that serve to protect that cell from subsequent increases in temperature.1 A group of proteins known as the heat shock proteins (HSPs) are the major proteins synthesized during such stress and play a pivotal role in providing this protection.2,3 A similar increase in stress protein synthesis is seen after a variety of nonthermal stresses; for example, in myocardial tissue, stress protein synthesis is increased by ischemic and mechanical injury.4-6 It seems likely, therefore, that stress proteins are involved in recovery from nonthermal as well as thermal injury. This conclusion is supported by the fact that stress proteins raised by one form of sublethal injury seem capable of protecting against a subsequent but different injury, a phenomenon known as cross-tolerance.7 In keeping with this phenomenon, a number of investigators have demonstrated that whole-body hyperthermia increases myocardial stress protein content and renders the isolated blood- or buffer-perfused heart resistant to subsequent ischemia and reperfusion.8-10 However, our attempts to use whole-body heat stress to limit infarction after a 45-minute coronary ligation were successful in the isolated blood-perfused heart11 but unsuccessful in vivo,12 leading to the speculation that whole-body heat stress may have deleterious aspects that negate any beneficial effects secondary to myocardial stress protein elevation.11 In contrast, in the in situ rat heart, Donnelly and coworkers13 have demonstrated that whole-body heat stress can reduce infarct size after a 35-minute but not a 45-minute coronary occlusion.

The purpose of this study was to examine in more detail the relation between myocardial stress protein content and the subsequent resistance of the in situ rabbit heart to ischemia. An established protocol4 of repetitive short coronary artery occlusions was used to selectively elevate myocardial stress protein content, thereby avoiding whole-body heat stress and its possible deleterious consequences. More importantly, brief coronary occlusions, unlike thermal stress, are a physiologically more relevant stimulus for stress protein induc-
Group 3, heat stress, consisted of 16 rabbits, with 2 exclusions. One rabbit died during the 30-minute coronary ligation, and 1 experiment had to be excluded because of inadequate staining of viable myocardium with triphenyltetrazolium. Ten rabbits were used for infarct size assessment and 4 rabbits for stress protein estimation.

Group 4, sham heat stress, consisted of 15 rabbits. There was 1 exclusion because of inadequate tissue staining. Ten rabbits were used for infarct size assessment and 4 rabbits for stress protein estimation.

Ischemic and Sham Ischemic Pretreatments

Rabbits were anesthetized with intramuscular fentanyl 100 μg/kg and fluanisone 3 mg/kg (Janssen Pharmaceuticals, Oxford, UK), followed by intraperitoneal diazepam 2 mg/kg. Anesthesia was maintained by fentanyl and fluanisone administered every 30 minutes. Once the rabbits were adequately anesthetized, they were orally intubated, and limb lead ECG electrodes (Medicotest, Rugmarken, Denmark) were attached. The rabbits were mechanically ventilated with 100% oxygen at a tidal volume of 5 mL/kg delivered at a rate of 1 Hz. A marginal ear vein was cannulated to administer fluids and drugs, and 30 mg of intramuscular amoxycillin was given prophylactically.

The heart was exposed through a median sternotomy, and a coronary artery (usually an anterolateral branch of the circumflex) was underrun with 3/0 silk suture on a tapered needle. The free ends of the suture were passed through a soft vinyl tube so as to form a snare to occlude the artery. Rabbits then received 1000 units of heparin before the first 5-minute coronary artery ligation. Successful ligation was confirmed by myocardial blush and resolution of the amplified ECG signal (ECG amplifier and series 3000 recorder, Gould Inc, Cleveland, Ohio). Reperfusion was confirmed by a myocardial blush and resolution of ECG changes. After four 5-minute coronary ligations separated by 10 minutes of reperfusion, the vinyl occluder was removed, and the coronary suture was left in situ while the sternotomy was closed by suturing muscle and then skin layers. Animals were given 10 mL/kg of 0.9% saline intravenously, allowed to breathe spontaneously, and eventually extubated.

The surgical preparation for the sham ischemic pre-treatment group was identical to that described above. However, although the pericardium was opened, a coronary artery was neither underrun nor occluded, thus avoiding mechanical manipulation of the myocardium, which may in itself act as a trigger for stress protein induction.

Heat Stress and Sham Heat Stress Pretreatments

Rabbits were anesthetized with pentobarbitone 40 mg/kg delivered via a marginal ear vein. In the heat-stressed group, rectal temperature was raised to at least 42°C for 15 minutes by wrapping the anesthetized rabbit in an electric warming blanket. Animals were then allowed to recover at room temperature.

Sham heat stress rabbits were identically anesthetized and wrapped for similar periods with the blanket not turned on.
Stress Protein Estimation

Twenty-four hours after the pretreatment intervention, rabbits were reanesthetized with intravenous pentobarbitone (60 mg/kg), and hearts were removed for stress protein determination (see Fig 1). Excised hearts were washed and briefly retrogradely perfused with iced saline to remove blood and albumin. In the ischemic pretreatment group, the coronary tie was retightened and Coomassie brilliant blue R250 dye (BDH Chemicals, Poole, England) was introduced into the coronary perfusate. Hearts were then removed from the perfusion rig, and atria, fat, and right ventricular free wall were removed. In the ischemic pretreatment group, the area without dye (risk zone) was separated from stained (perfused) myocardium. Left ventricular specimens were then rapidly frozen in liquid nitrogen.

At a later date, myocardial specimens were crushed and homogenized in 2× concentrated sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (20% glycerol and 6% SDS in 0.12 mol/L Tris at pH 6.8), and protein concentrations were estimated by use of the Pierce BCA reagent (Pierce, Rockford, Ill) and equalized by further addition of sample buffer where necessary; 2-mercaptoethanol to a final concentration of 10% was added before boiling. Samples were then centrifuged and stored at −70°C. Subsequently, samples were thawed, recentered, and further diluted in sample buffer to allow loading of approximately 30 μg of total protein per lane of slab gel.

Proteins were separated by SDS-PAGE on 0.8-mm-thick, 12.5% acrylamide gels according to Laemmli. Equivalence of loading and adequacy of sample preparation were determined by visualization of proteins with Coomassie blue stain. When loading conditions were optimal, three identical gels were prepared, each loaded with four heart samples derived from each of the four groups. The proteins on two gels were transferred onto a nitrocellulose membrane (Hybond C, Amersham, Bucks, UK) by Western blotting. The other identical gel was stained with Coomassie brilliant blue to visualize protein for subsequent densitometry.

One nitrocellulose membrane was washed in phosphate-buffered saline with 0.1% dried skimmed milk powder to block nonspecific binding sites. After washing, the membrane was incubated at 37°C for 1 hour with mouse monoclonal IgG cross-reactive to the inducible 72-kD heat shock protein (Stressgen, Sidney, Canada) at 1:1000 dilution. After repeated washing, the membrane was incubated with horseradish peroxidase–conjugated rabbit anti-mouse IgG (DAKO, Denmark) at 1:1000 dilution at room temperature for 1 hour. The filter was then washed and developed by use of enhanced chemiluminescence detection (Amersham, Bucks, UK).

The other identical nitrocellulose membrane was blocked with 3% skimmed milk powder and after washing at room temperature for 3 hours was exposed to a mouse monoclonal IgG raised against human heat shock protein 60 at 1:2000 dilution. Subsequent methods were as described above with the second antibody at 1:2000 dilution. The monoclonal used recognizes only the mitochondrial form of the 60-kD stress protein and does not cross-react with the cytosolic homologue (TCP1).

The relative levels of heat shock proteins 72 and 60 were determined using densitometry (model 620 videodensitometer with Biorad analyst 2 version 3.1 software, Biorad, Hemel Hempstead, UK), normalizing to the actin band on the Coomassie-stained gel. This procedure adjusts for slight variations in protein loading between samples.

Myocardial Infarction and Infarct Size Assessment

Approximately 24 hours after the above pretreatments, animals were reanesthetized with pentobarbitone (40 mg/kg) via the marginal ear vein. ECG electrodes were attached, and the trachea was opened and intubated via a midline cervical incision. Mechanical ventilation was as previously described; however, the tidal volume was reduced to 4 mL/kg in keeping with the reduction in ventilatory dead space. The right common carotid artery was cannulated with a short rigid polyethylene cannula attached to a pressure transducer (P23XL, Gould) for continuous recording of arterial pressure and intermittent arterial blood gas estimations (ABL2, Radiometer, Copenhagen, Denmark). Throughout the procedure, rectal temperature was monitored and maintained between 38.5°C and 39.0°C by an electric warming pad. The chest was opened (or reopened) via a midline sternotomy, and a coronary artery was identified. Rabbits were given 1000 units of heparin intravenously, and the coronary artery was ligated as described for the ischemic pretreatment group. In the case of the ischemic pretreatment group, the existing coronary tie was reused to ensure that the same coronary bed was rendered ischemic as on the previous day. In the sham ischemia group, identification of a suitable coronary vessel was often difficult, since the surface of the heart was ensased in a thin fibrinous exudate.

After a 30-minute coronary occlusion, reperfusion was confirmed by the appearance of a myocardial blush. After 120 minutes of reperfusion, a further 1000 units of heparin were given, and the heart was removed and retrogradely perfused with 0.9% saline. After blood had been washed out of the coronary vasculature, the coronary snare was retightened and fluorescent microspheres were infused via the aortic cannula. Demarcation of the myocardial surface at risk (area without spheres) was confirmed under UV light. The heart was then frozen at −18°C overnight. The next day, while still frozen, the heart was sliced at 2-mm intervals at right angles to its long axis. The slices were then incubated at 37°C in triphenyltetrazolium chloride 10 mg/mL of phosphate buffer at pH 7.4. When the viable myocardium had stained, slices were placed in 10% formaldehyde solution. Approximately 24 hours later, heart slices were placed caudal surface upward and compressed between two glass plates separated by 2-mm spacers. Risk areas and areas of infarction were traced, photographically enlarged, and planimetered (Summa Graphics, Summa Sketch II, Seymour, Conn). The area of infarction (no tetrazolium staining) was expressed as a percentage of the area at risk of infarction (no fluorescent microspheres). The volume of myocardial tissue at risk and the volume of infarction were calculated by
multiplying the corresponding areas by the depth (2 mm) of the tissue slices.

Statistics

All values are expressed as mean±SEM. All comparisons between groups were assessed for significance with a one-way ANOVA, Fisher’s protected least significant difference method being used for comparisons within the ANOVA table. Changes in hemodynamic parameters within groups over the course of the experiment were compared by two-way ANOVA with a post hoc paired t test for comparison of variables at baseline and end of reperfusion. An unpaired t test was used to compare baseline temperatures between heat stress and sham heat stress groups. Association between rate-pressure product (RPP) and infarct size was tested by the Spearman rank correlation method. Statistical significance was defined as P≤.05.

Results

Temperature Changes During Heat Stress

Before warming, there was no significant difference between basal rectal temperature within sham or heat stress groups (39.1±0.1°C, n=14, and 39.0±0.1°C, n=14, P=NS, respectively). After the heat stress rabbits were wrapped in an electric warming blanket, an average of 42.6±2.9 minutes elapsed before the rectal temperature reached 42°C. The peak temperature recorded was 42.3±0.1°C, and the time that the rectal temperature remained above 42°C was 17.6±1.4 minutes.

Myocardial 72-kD Stress Protein Content

An initial protocol with only 6 hours of recovery after ischemic pretreatment caused some increase in HSP72, but this was not significantly different from basal expression and was significantly less than the elevation seen 24 hours after heat stress. The recovery time after ischemic pretreatment was therefore increased to 24 hours. Fig 2 demonstrates that significant induction of the 72-kD stress protein occurred 24 hours after heat stress and ischemic pretreatments. This protein was detected in all myocardial samples examined (including those from the nonischemic area, data not shown). When the blot was examined densitometrically, the graded induction of stress protein with differing interventions became more apparent with thermal pretreatment, resulting in marginally greater induction than ischemic pretreatment. However, the mean stress protein content in each of the pretreatment groups was greater than in corresponding controls. Between the control groups, there was a significant induction in the sham ischemic pretreatment group. The reason for this may lie in the greater surgical trauma occurring with sham ischemic pretreatment, although other differences exist between these two control groups, including the method of ventilation and anesthetic regimens. Compared with the sham heat stress group, there was an approximately eightfold HSP72 induction by thermal, sevenfold by ischemic, and threefold by sham ischemic pretreatments.

Myocardial 60-kD Stress Protein Content

Fig 3 demonstrates that the marked variation seen between experimental groups for the 72-kD stress protein was not apparent when an identically loaded gel was blotted and probed for the 60-kD stress protein. In this model, cardiac HSP60 was not significantly elevated by whole-body heat stress. The only intervention associated with any change in HSP60 content was ischemic pretreatment, which resulted in an approximately 1.5- to 2-fold induction compared with other intervention groups. Therefore, differences exist in stress protein inducibility, with HSP72 being more inducible than HSP60, whereas HSP60 is preferentially, though only modestly, induced by ischemia.

Infarct Size

Risk volume, infarct volume, and percentage of risk area infarcted are shown in Fig 4. The volumes of myocardial tissue at risk after coronary ligation were not significantly different between intervention groups. However, thermal or ischemic pretreatment resulted in both a significant reduction in absolute infarct volume and a reduction in infarct volume expressed as a percentage of the volume of tissue at risk. The volume of infarction as a percentage of the volume at risk for
ischemic pretreatment compared with control was 28.8±5.2% vs 52.0±5.2%, $P \leq .01$, respectively, and for thermal pretreatment compared with control, 32.8±3.8% vs 56.9±6.5%, $P \leq .01$, respectively. Therefore, both ischemic and thermal pretreatment resulted in myocardial protection.

In all treatment groups, the infarcted areas exhibited intramyocardial hemorrhage. The absence of any hemorrhage was rare and usually indicative of a failure to reperfuse (see exclusions).

**Hemodynamic and Metabolic Data**

Fig 5 describes the changes in RPP that occur before, during, and after the 30-minute coronary occlusion for each group. There were no differences in RPP between groups before coronary occlusion or during ischemia, implying that myocardial work and therefore oxygen demand was likely to be similar between groups.

During reperfusion, the RPP was consistently higher in the heat stress group, and at various time points, it was significantly different from all other groups. This preservation of RPP is likely to be independent of infarct size, since a similar reduction in infarct size was seen with ischemic pretreatment but was not associated with any significant advantage in RPP. In an attempt to further elucidate the reason for this apparent advantage, we correlated RPP at the end of reperfusion with infarct volume as a ratio of heart mass (volume to heart weight ratio). As seen in Fig 6, there was no correlation between infarct size and RPP for any group. This finding suggests, surprisingly, that in this experimental model, infarct size does not appear to be the major determinant of cardiac work.

The Table includes the hemodynamic as well as acid/base balance data for each experimental group (the RPP is displayed in graphic form in Fig 5). There were no significant differences between groups in terms of acid/base balance or heart rate.

**Discussion**

This study demonstrates that both ischemic and heat stress pretreatments elevate myocardial HSP72 to a similar extent and are associated with a similar reduction of infarct size. This reduction occurs despite similar RPPs before and during prolonged coronary artery occlusion and therefore suggests that the protection is a direct result of enhanced myocardial resistance to infarction. This enhanced resistance to infarction is at

**FIG 3. Densitometric assessment of Western blot loaded with four specimens from each of the four intervention groups and probed against heat stress protein (HSP)60. The lane arrangement gel loading conditions and figure layout are identical to Fig 2. HSP60 levels appeared quite similar between groups. The HSP60 band did, however, appear denser in the lanes containing samples from the ischemically pretreated hearts. Densitometry confirmed that ischemic pretreatment was associated with a selective induction of HSP60. Optical density ratios, ischemic pretreatment vs sham ischemia, heat stress, and sham heat stress were 3.8±0.4 vs 2.4±0.3, 2.6±0.3, and 2.1±0.2, respectively. All comparisons were by ANOVA.**

**FIG 4. Bar graphs of risk and infarct zone volumes. Risk zones were demarcated by the absence of fluorescent microspheres and infarct zones by absence of staining with triphenyltetrazolium. Both ischemic and heat stress pretreatments were associated with a significant reduction in infarct volume as well as infarct zone as a percentage of risk zone. All comparisons were by ANOVA.**
least associated with HSP72, and it is tempting to postulate that it occurs as a direct consequence of myocardial stress protein induction.

**Basis for the Reduction in Myocardial Infarct Size**

The design of this study is similar to and the findings are consistent with those of Donnelly et al. Those investigators were able to demonstrate significant infarct size reduction after whole-body heat stress; however, ischemic pretreatment with a single 20-minute coronary occlusion failed to reduce infarct size but also failed to increase myocardial HSP72 to the level seen after heat stress. Those investigators, therefore, concluded that the absolute levels of HSP72 may be important in conferring protection from ischemic injury. This conclusion is supported and extended by the findings of our study, which demonstrate that greater ischemic elevation of myocardial HSP72 is indeed associated with protection. Moreover, a modest elevation of HSP72, as occurred with sham ischemic pretreatment, is not associated with protection. In addition, our study suggests that HSP60 induction appears not to be a prerequisite for myocardial salvage, whereas an appropriate level of HSP72 may be. However, the relation between whole-body heat stress and subsequent myocardial salvage is complicated by the fact that in the isolated rat heart, protection seems to involve an increase in myocardial catalase activity rather than an induction of HSP72, since protection can be abolished by inhibition of myocardial catalase with 3-amino-triazole. In a preliminary report, endogenous catalase activity was also increased by repeated ischemia and reperfusion; therefore, a common

---

**FIG 5.** Graph of rate-pressure product before, during, and after 30-minute coronary occlusion. The shaded area represents the period of coronary occlusion. Although no significant differences existed before or during coronary occlusion, upon reperfusion, rate-pressure product was consistently higher in the heat stress group. Heat stress was the only intervention without a significant drop in rate-pressure product for the duration of the experiment. The minus-15-minute hemodynamic data were collected while the chest was closed, and the minus-5-minute data were collected when the chest and pericardium were reopened. *P ≤ 0.05 heat stress vs ischemia and sham ischemia; †P ≤ 0.05 heat stress vs sham ischemia and sham heat stress; ##P ≤ 0.05, ###P ≤ 0.01 for each group at 150 minutes vs baseline by ANOVA.

**FIG 6.** Scatterplots showing relation between infarct volume normalized to heart weight and rate-pressure product at 120 minutes of reperfusion. R² represents the coefficient of determination. There is no correlation between normalized infarct volume and rate-pressure product in any of the intervention groups. This finding implies that infarct volume does not determine rate-pressure product in our experimental model.
### Hemodynamic, Arterial pH, and Oxygen Tension Changes During Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Preocclusion</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>297±6</td>
<td>277±7</td>
<td>272±9</td>
<td>278±6</td>
<td>270±5</td>
<td>257±6</td>
<td>258±7</td>
<td>255±5</td>
<td>269±13</td>
<td>271±11</td>
</tr>
<tr>
<td>Sham ischemia</td>
<td>293±11</td>
<td>277±9</td>
<td>271±10</td>
<td>271±11</td>
<td>284±15</td>
<td>267±10</td>
<td>271±8</td>
<td>268±10</td>
<td>270±11</td>
<td>260±14</td>
</tr>
<tr>
<td>Heat stress</td>
<td>273±9</td>
<td>273±10</td>
<td>269±9</td>
<td>269±8</td>
<td>265±9</td>
<td>256±10</td>
<td>259±10</td>
<td>266±12</td>
<td>262±11</td>
<td>264±11</td>
</tr>
<tr>
<td>Sham heat stress</td>
<td>281±7</td>
<td>277±10</td>
<td>270±13</td>
<td>265±14</td>
<td>272±12</td>
<td>263±11</td>
<td>266±11</td>
<td>264±9</td>
<td>261±10</td>
<td>260±9†</td>
</tr>
<tr>
<td><strong>Arterial systolic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>92±7</td>
<td>90±7</td>
<td>76±10</td>
<td>77±6</td>
<td>74±6</td>
<td>70±5</td>
<td>70±6</td>
<td>67±6</td>
<td>73±6</td>
<td>74±8‡</td>
</tr>
<tr>
<td>Sham ischemia</td>
<td>91±6</td>
<td>76±4</td>
<td>67±4</td>
<td>72±3</td>
<td>75±6</td>
<td>68±6</td>
<td>68±5</td>
<td>67±4</td>
<td>65±5</td>
<td>71±6</td>
</tr>
<tr>
<td>Heat stress</td>
<td>99±6</td>
<td>86±6</td>
<td>83±6</td>
<td>87±6</td>
<td>84±7</td>
<td>85±6.4*</td>
<td>89±6</td>
<td>94±6†</td>
<td>88±6‡</td>
<td>87±6‡</td>
</tr>
<tr>
<td>Sham heat stress</td>
<td>92±6</td>
<td>86±5</td>
<td>78±6</td>
<td>82±7</td>
<td>81±5</td>
<td>75±5</td>
<td>73±5</td>
<td>78±5</td>
<td>61±6</td>
<td>63±7†</td>
</tr>
<tr>
<td><strong>Arterial diastolic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>73±7</td>
<td>71±8</td>
<td>59±9</td>
<td>60±6</td>
<td>56±6</td>
<td>52±5</td>
<td>52±5</td>
<td>49±5</td>
<td>55±6</td>
<td>55±8‡</td>
</tr>
<tr>
<td>Sham ischemia</td>
<td>73±6</td>
<td>59±4</td>
<td>53±4</td>
<td>58±3</td>
<td>59±5</td>
<td>53±6</td>
<td>52±5</td>
<td>50±4</td>
<td>50±5</td>
<td>52±6‡</td>
</tr>
<tr>
<td>Heat stress</td>
<td>81±7</td>
<td>73±7</td>
<td>70±6</td>
<td>75±6</td>
<td>67±8</td>
<td>69±7</td>
<td>74±6*</td>
<td>76±5*</td>
<td>72±7</td>
<td>71±6</td>
</tr>
<tr>
<td>Sham heat stress</td>
<td>78±7</td>
<td>70±5</td>
<td>63±7</td>
<td>68±8</td>
<td>65±7</td>
<td>62±8</td>
<td>57±7</td>
<td>58±6</td>
<td>43±6</td>
<td>49±6‡</td>
</tr>
<tr>
<td><strong>Mean arterial pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>81±7</td>
<td>80±8</td>
<td>66±9</td>
<td>68±6</td>
<td>63±6</td>
<td>60±5</td>
<td>60±6</td>
<td>56±5</td>
<td>62±7</td>
<td>63±8‡</td>
</tr>
<tr>
<td>Sham ischemia</td>
<td>79±5</td>
<td>66±4</td>
<td>59±4</td>
<td>64±4</td>
<td>65±5</td>
<td>59±6</td>
<td>60±5</td>
<td>56±4</td>
<td>57±5</td>
<td>58±6‡</td>
</tr>
<tr>
<td>Heat stress</td>
<td>88±6</td>
<td>80±7</td>
<td>77±6</td>
<td>80±6</td>
<td>76±7</td>
<td>77±7</td>
<td>82±6*</td>
<td>84±6†</td>
<td>79±7‡</td>
<td>78±6</td>
</tr>
<tr>
<td>Sham heat stress</td>
<td>84±7</td>
<td>76±4</td>
<td>74±8</td>
<td>75±7</td>
<td>76±6</td>
<td>68±6</td>
<td>68±6</td>
<td>71±6</td>
<td>56±6</td>
<td>60±7‡</td>
</tr>
<tr>
<td><strong>Arterial blood pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>7.49±0.02</td>
<td>7.50±0.03</td>
<td>7.46±0.02</td>
<td>7.45±0.02</td>
<td>7.47±0.02</td>
<td>7.44±0.02</td>
<td>7.44±0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham ischemia</td>
<td>7.53±0.02</td>
<td>7.48±0.02</td>
<td>7.43±0.04</td>
<td>7.44±0.02</td>
<td>7.43±0.01</td>
<td>7.40±0.02</td>
<td>7.39±0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat stress</td>
<td>7.51±0.02</td>
<td>7.53±0.04</td>
<td>7.46±0.02</td>
<td>7.44±0.02</td>
<td>7.45±0.01</td>
<td>7.45±0.01</td>
<td>7.45±0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham heat stress</td>
<td>7.46±0.02</td>
<td>7.45±0.04</td>
<td>7.41±0.03</td>
<td>7.42±0.03</td>
<td>7.42±0.02</td>
<td>7.38±0.04</td>
<td>7.38±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arterial blood oxygen (kPa)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>47.9±6.7</td>
<td>51.3±5.8</td>
<td>48.9±5.6</td>
<td>47.2±6.1</td>
<td>47.8±4.7</td>
<td>50.7±5.3</td>
<td>54.2±4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham ischemia</td>
<td>60.3±3.1</td>
<td>59.1±3.1</td>
<td>55.1±2.0</td>
<td>51.4±4.7</td>
<td>48.4±4.6</td>
<td>50.1±3.5</td>
<td>54.9±3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat stress</td>
<td>61.5±4.2</td>
<td>58.9±2.3</td>
<td>56.3±3.3</td>
<td>59.4±4.0</td>
<td>55.9±4.0</td>
<td>59.6±2.3</td>
<td>59.5±1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham heat stress</td>
<td>52.3±5.8</td>
<td>54.7±2.0</td>
<td>50.6±2.6</td>
<td>53.1±2.0</td>
<td>50.5±3.4</td>
<td>50.5±5.0</td>
<td>55.0±6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*n=10 for each group. bpm. Beats per minute. Baseline data were recorded before the chest was opened. Mean weights (kg): ischemia, 2.57±0.14; sham ischemia, 2.40±0.12; heat stress, 2.58±0.22; sham heat stress, 2.45±0.16.

*P≤.05, †P≤.01 vs ischemia and sham ischemia; ‡P≤.05, §P≤.01 vs sham ischemia and sham heat stress using ANOVA; ¶P≤.05, ¶¶P≤.01 for each individual group at 150 minutes vs baseline using ANOVA.

The mechanism independent of stress proteins may underlie the protection seen with both ischemic and heat stress pretreatments. Somewhat against this explanation is the observation that heat stress is protective in a number of different biological systems against injuries not obviously involving oxidant stress. More importantly, a recent report suggests that heart cells transfected so as to overexpress HSP72 become resistant to anoxic injury. A possible explanation for these apparently contradictory findings is the suggestion that stress proteins may in some way modify catalase activity. This is supported by the observation that the increase in myocardial catalase after heat stress is not transcriptionally regulated.

The mechanisms whereby stress proteins may directly limit infarct size are necessarily speculative, since at the molecular level, the key sites of injury during ischemia are poorly understood. The most attractive possibilities involve the stabilization of the cytoskeleton during ischemia by α-crystallin (an HSP homologue). Alternatively, the chaperoning function (of the HSP90 and HSP70 families) of stress proteins may allow the correct refolding of proteins sublethally damaged by ischemia/reperfusion or may prevent inappropriate molecular interactions between these damaged proteins and otherwise viable proteins. A hypothesis in keeping with the finding that cell turnover is lower in myocyte cultures that overexpress HSP72 and are resistant to simulated ischemia.

In the ischemic pretreatment group, the protection observed could be an ischemia-triggered increase in collateral flow or stunning. Both these explanations are unlikely, since it is unclear whether the oxygen requirements and susceptibility to infarction are increased, decreased, or normal in stunned myocardium. In a previous study, a pattern of ischemic pretreatment...
identical to that used in our experiments produced only a very mild degree of stunning. In addition, the rabbit is a species deficient in collateral vessels; therefore, any increase in collateral blood flow occurring as a result of ischemic pretreatment would have to be on the basis of new (mitotic) vessel growth, and this is unlikely to occur over a 24-hour period.

Relation Between Ischemic Pretreatment and Ischemic Preconditioning

The protocol used in this study and protocols known to trigger “ischemic preconditioning” are identical. Ischemic preconditioning, however, is a short-lived phenomenon with protective benefits waning within 2 hours of reperfusion. This study therefore raises the possibility of a delayed, but perhaps longer-lasting, second window of protection (SWOP) (a biphasic protective effect). Evidence in support of such a phenomenon exists in neuronal tissue, since the stress protein content of neuronal tissue can be elevated by 2-minute, daily episodes of ischemia, a treatment that also seems to increase the neuronal resistance to more prolonged ischemia. Preliminary findings from other laboratories confirm a similarly delayed protection at 24 hours after “classic” preconditioning of the dog heart (with repeated coronary occlusions) and also 24 hours after rapid pacing of the rabbit heart. These experimental findings may explain the apparent benefit of a ≥7-day history of angina before myocardial infarction, although differences in treatment and collateral vessel formation may complicate the issue.

The relation between the early phase of protection after brief episodes of ischemia (as seen in ischemic preconditioning) and the later phase of protection (as seen in this study) is speculative. An obvious inconsistency is the fact that multiple coronary occlusions are as effective as a single occlusion in triggering preconditioning but differ in their ability to induce myocardial HSP72. This inconsistency may explain why protection against ischemic arrhythmia was not seen 24 hours or 72 hours after a single 5-minute coronary occlusion in the rat.

Conclusions

The results of this study indicate that substantial myocardial HSP72 induction is possible with either sublethal thermal or ischemic pretreatment, whereas HSP60 is preferentially elevated by ischemic pretreatment. This observation is the first indication that myocardial HSP72 may be more important than other stress proteins, since myocardial salvage was seen after a 30-minute coronary occlusion with both ischemic and thermal pretreatments. In addition to limiting infarct size, whole-body heat stress has other nonspecific effects that act to maintain RPP.

Our results provide evidence for what may prove to be a clinically relevant myocardial adaptive response occurring 24 hours after short episodes of cardiac ischemia that serves to increase myocardial resistance to infarction.

Acknowledgments

The anti-60-kD heat shock protein antibody was a gift from Dr G.A. Rook, Department of Medical Microbiology, University College London Medical School. M.S.M. is the recipient of an intermediate fellowship from the British Heart Foundation. We thank the Hatter Foundation for continued support.

References


Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction.
M S Marber, D S Latchman, J M Walker and D M Yellon

Circulation. 1993;88:1264-1272
doi: 10.1161/01.CIR.88.3.1264

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/88/3/1264

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/